Sex Differences in Attentional Bias before and after Stress Induction: An Event Related Potential Study

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Statement of Sources

I declare that this report is my own original work and that contributions of others have been duly acknowledged.

Date: Signature:

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Abstract

Females have double the lifetime prevalence of anxiety disorders compared to males. Attentional bias to threat and arousal reactivity have been consistently implicated as a potential mechanism underlying the development and maintenance of anxiety disorders. Sex differences in attentional bias and arousal may contribute towards the prevalence for anxiety disorders in females. There is limited research exploring sex differences in attentional bias, and the literature is inconsistent, possibly due to relying on RT as a dependent measure for attentional bias, and not accounting for baseline arousal. One recent study found females displayed increased arousal and attentional bias to threat following acute stress induction. To replicate and extend these findings ERPs, RT and salivary alpha amylase (sAA, indexed noradrenaline) were measured to examine sex differences in P1 and N1 (reflecting early visual orientation) and P3 component (reflecting conscious allocation of visual resources). sAA results indicated that acute-stress induction produced significant increase in stress hormone noradrenaline, but females did not have heighted arousal reactivity. RT and ERP component analysis indicated no attentional bias to threat in females or males. These findings did not confirm the predictions of the study. The limitations of the present study and future research suggestions are also discussed.

Large scale epidemiological research has established that females have approximately double the lifetime prevalence rates for anxiety disorders, such as posttraumatic stress, panic disorder, and agrophobia, compared to their male counterparts (Kessler, Chia, Emler, Merikangas & Walters, 2005). The mechanisms that underlie female vulnerability for anxiety disorders remains relatively unknown (Tolin & Foa, 2006). Two key mechanisms that have been suggested to influence the development and maintenance of anxiety disorders include attentional bias to threat (Cisler & Koster, 2010) and heightened sympathetic arousal in response to threat (Gorman, Kent, Sullican & Coplan, 2000). As such, it may be that attentional bias to perceived threat and heightened arousal are involved in female anxiety vulnerability (Cisler & Koster, 2010; Catuzzi & Beck, 2014). Despite the striking sex differences in anxiety disorder prevalence, sex is not consistently assessed in research investigating mechanisms contributing to the development and maintenance of anxiety disorders (Sass, Heller, Stewart, Silton, Edgar., et al., 2009). Considering this information, the exploration of sex differences in attentional bias and heightened arousal to threat is warranted.

Attentional Bias

Attentional bias is defined as a propensity to disproportionately assign greater attention to salient threatening stimuli, rather than neutral, in the environment (Kappenman, Farren, Luck & Proudfit, 2014). Cisler and Koster (2010) highlight three components that constitute attentional bias to threat; facilitated attention, difficulty in disengagement and attentional avoidance. Facilitated attention refers to quicker recognition of threatening stimuli compared to neutral stimuli within the environment.

Difficulty in disengagement is defined as finding it harder to remove attention away from a threatening stimuli, compared to non-threatening stimuli and finally, attentional avoidance is the allocation of attention to places in the environment away from the threat (Koster, Crombez, Verschuere, Van Damme & Wiersema, 2005; Mogg, Bradley, Miles, & Dixon 2004). The amalgamation of the research identifying these components of attentional bias has led to the development of several models of attentional bias to threat, including that developed by Bar-Haim. Lmay, Pergamin, Bakermans-Kranenburg and Van Ijzendoorn (2007).

Model of Attentional Bias

In a meta-analysis Bar-Haim, et al. (2007) proposed an integrative model of attentional bias to threat which emphasised the role of sympathetic arousal in attention (summarised in Figure 1). The model proposes four threat processing steps; the pre-attentive threat evaluation system (PTES), resource allocation system (RAS), a guided threat evaluation system (GTES) and the goal engagement system (GES). These four steps work to assess if an environmental stimulus is a threat, based on previous experience, and formulate an appropriate course of action to address any threat using available coping resources (summarised in Figure 1.).

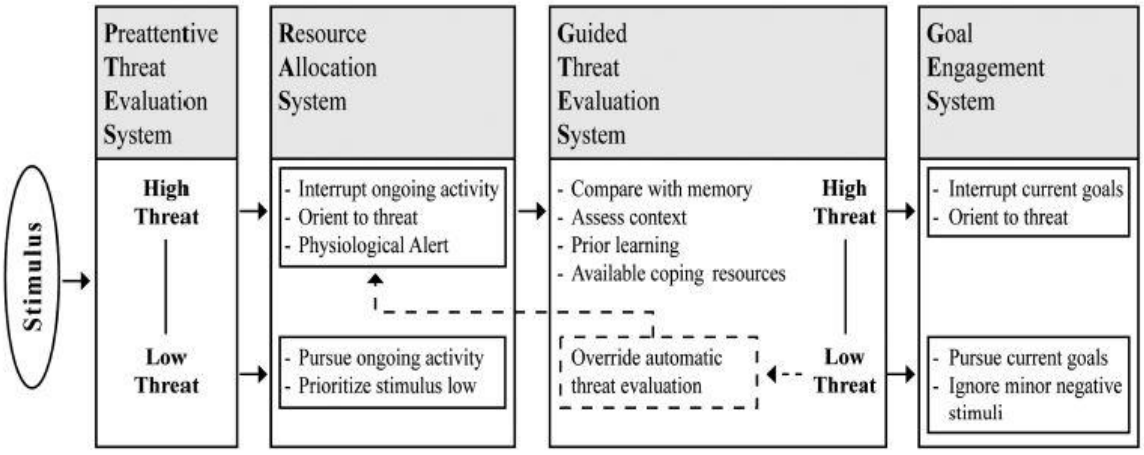

Figure 1. Model of the cognitive mechanisms underlying the processing of threat (Bar-Haim, et al. 2007).

The PTES is the earliest step in the model. Bar-Haim and colleagues propose that this step is facilitated by unconscious attentional processes which evaluate salient environmental stimuli. This is then communicated to the RAS, which initiates physiological arousal and the pre-conscious allocation of cognitive resources to stimuli deemed to be a threat (this is synonymous with the attentional bias component facilitated attention). Threatening stimuli will lead to difficulty disengaging attention away which over time can result in high states of anxiety. Persistent anxious states are believed to be prolonged in individuals with an attentional bias to threat. Finally the GTES is triggered and conscious attentional processes are activated. Utilizing past experience, context and relevance of threat, the stimuli will be deemed as either high- or low- threat. If the stimuli is classified as low-threat, it is ignored and physiological arousal is overridden by the GES allowing for resumption of normal functioning. If classified as high-threat, the GES focuses the attention to threat by impeding current-task orientated behaviours. Notably, Bar Haim's model integrates sympathetic arousal

into attentional bias, and a convergent model also proposes a link between noradrenergic arousal and biased attention to salient stimuli.

Biased Attention via Norepinephrine

Stemming predominantly from animal research, Markovic, Anderson and Todd (2014) proposed that a central mechanism modulating attentional bias to salient stimuli is increased activity of stress hormone norepinephrine, stemming from the locus coeruleus. These amplified activation cues cause changes in the visual cortex, leading to an increase in the subjective experience of emotionally salient stimuli. This model has been labelled the Biased Attention via Norephinephrine[1] (BANE) model which combines genetics, neuromodulatroy, neural and behavioural components which each explain some of the variance in attentional bias. The model focuses on the noradrenergic processes in the anterior affective system, including the amygdala and orbitofrontal cortex; both areas are implicated in directing attention to emotion evoking stimuli (Rudrauf, David, Lachaux, Kovach, Martimerie, et al., 2008). Convergent evidence is found in animal studies using single-electrode studies. Aston-Jones, Rajkowkis, & Cohen (1999) discovered that increased arousal reactivity, in particular, noradrenergic system activation in the locus coeruleus, led to orientation toward salient, threatening stimuli in the environment. To summarize, the BANE model implicates the role of noradrenaline in attentional bias to salient, negative and threatening stimuli. Given these convergent models highlighting the role of sympathetic arousal in attentional bias, one possible explanation of female vulnerability for the development of anxiety disorders is heightened arousal reactivity, which may then lead to greater attentional bias.

[1] Norepinephrine is also known as noradrenaline and hereafter will be referred to as noradrenaline.

Sex Differences and Arousal

Anxiety disorders such as PTSD, Panic Disorder and Specific Phobia, all characteristically present with symptoms of increased sympathetic and somatic arousal in response to threat (Southwick, Bremner, Rasmusson, Morgan, Arnsten & Charney, 1999; Gorman, Kent, Sullivan & Coplan, 2000). It is known that the sympathetic arousal experienced as part of these disorders are moderated, in part, by noradrenergic activity. Noradrenergic activity originates in the locus coeruleus which is part of the pontine brainstem (Aston-Jones, et al., 1999). Physiological manipulations of this locus coeruleus activity can result in changes to sympathetic arousal (Aston-Jones, Rajkowski & Cohen, 1999). Anxiety disorders, in particular PTSD and Panic Disorder, have been shown to have heightened noradrenergic activity (Southwick et al., 1999; Gorman et al., 2000). Neuroimaging research has further supported this. Individuals with PTSD, Social Anxiety and Specific Disorder all demonstrated increased activity in the amygdala, a control centre for sympathetic arousal, which receives a significant amount of noradrenergic brainstem afferents (Aston-Jones et al., 1999; Etkin & Wager, 2007). Furthermore, this increased activity to threat can be seen in brainstem arousal networks which comprise of noradrenergic afferents from the locus coeruleus in individuals with PTSD and panic disorder (Felmingham, Williams, Kemp, Liddell, Falconer, Peduto et al., 2010; Gorman, et al., 2000).

There is currently some evidence that suggests females display heightened physiological reactivity to threatening stimuli compared to males (Mclean & Anderson, 2009). Following a psychosocial stressor, symptoms such as increased blood pressure, resting heat rate and stress hormone reactivity, are observed to be significantly greater

in females compared to males (Back, Waldrop, Saladin, Yeatts, Simpson, McRae et al., 2008), however there is some inconsistency in the literature which may be due to the operationalization of physiological reactivity (Mclean & Anderson, 2009). Research by Segal and Cahill (2009) found that females display a greater noradrenergic response to emotional stimuli, measured by salivary alpha amylase (sAA), a reliable and known index of endogenous noradrenergic activity (Rohleder & Nater, 2009). In an fMRI study examining sex differences following trauma exposure, greater brainstem activity to threatening faces was observed in women with PTSD and following trauma exposure (Felmingham, et al., 2010). Felmingham et al., (2010) concluded that this heightened activity to threat may reflect a vulnerability factor for females developing PTSD.

Sex Differences and Attentional Bias

The dot-probe paradigm has long been considered the gold-standard task to measure attentional bias, and has consistently demonstrated attentional bias to threat in anxious individuals (Mogg & Bradley, 1998; Van Bockstaele, Verschuere, Tibboel, Houwer, Crombez, et al., 2013). Dot-probe tasks present a threatening stimulus and neutral stimuli simultaneously, followed by a target item (probe) in a cued location. There are typically two conditions; congruent and incongruent. In a congruent trial a probe is presented behind the threat stimuli and in an incongruent trials a probe is presented behind the neutral stimulus. In a paired trial, the participants need to identify on which side the probe was presented, as quickly and as accurately as possible. If reaction time (RT) is found to be significantly faster for identification of probe behind threat stimuli, compared to neutral stimuli, it is deemed to be evidence of an attentional bias to threat or facilitated attention (Bar-Haim, Lamy, Pergamin et al., 2007; Cisler &

Koster, 2010). If reaction time in congruent trials is significantly slower than incongruent trial it is judged to be attentional avoidance. Additionally, attentional bias is also inferred by decreased reaction time in incongruent trials which, is indicative of difficulties disengaging with the threatening stimuli (Koster, Crombez, Verschuere, & De Houwer, 2004; Cisler & Koster, 2010; Sagliano, Trojano, Amoriello Migliozzi & D'Olimpio, 2007).

There is limited evidence of sex differences in attentional bias, as there is a lack of studies that have addressed the question. In a spatial cueing task, high trait anxiety females demonstrated attentional bias towards emotionally aversive stimuli compared to neutral and pleasant scenes. Comparatively, low trait anxiety females tended towards attentional bias focussing their attention away from the threat. No effects of anxiety on attention were observed in males (Waters, Nitz, Craske, & Johnson, 2007). Using a probe detection paradigm Tan, Ma, Gao, Wu and Fang (2011) found that females exhibited difficulties disengaging attention away from locations that fearful faces were presented, unlike males who tended to avoid the location of the fearful faces. Angry facial expression also elicited an attentional bias in highly anxious females, with decreased reaction times in response to angry faces compared to happy faces, in a dot-probe task. Males in the same study exhibited a bias towards happy faces (Tran, Lamplmayr, Pintzinger & Pfabigan, 2013). Kreher, Powers and Granger (2012) found that healthy female participants had increased affective priming to negative words when noradrenergic levels were raised, further implicating noradrenaline in heightened processing of negatively valenced stimuli. These findings, in combination with research implicating arousal in the modulation of attentional bias warrants further exploration. A

stronger experimental design to assess sex differences in attentional bias would include the direct manipulation of arousal. A recent study that has examined attentional biases using a dot-probe task that was completed before and following an acute stress induction task (Carr, Scully, Webb, & Felmingham, 2015).

Using a dot-probe paradigm Carr, et al., (2015) examined sex differences in attentional bias prior to and following an acute stress induction via a cold pressor stress task. It has been found that the cold pressor stress tasks consistently increases noradrenaline levels (Mitchel, MacDonald, & Brodie, 2004). Participants were presented with pairs of human face images comprising of a neutral (happy or neutral) expression and a threat-related expression (fear, anger disgust). After the presentation of the stimuli, a probe appeared behind the target image which participants had to respond to. Using reaction time as a behavioural measure of attentional bias, if participants responded consistently faster to probes behind threat-related emotional faces this was considered evidence of attentional bias to threat, as per previous studies of a similar nature (Cisler & Koster, 2010; Mogg & Bradley, 1988). The results of Carr and Colleague's study suggested that females had greater arousal reactivity (indexed by noradrenaline) to the stress task, and a concomitant increase in attentional bias towards threat, following the stress task compared to the males who did not react to the acute stressor. Interestingly, they found that females exhibited an attentional avoidance at baseline. This finding was unexpected and requires replication.

However, research suggests the dot-probe tasks lack internal reliability and has limited test re-test reliability due to using reaction time as a dependent variable (Schmukle, 2005; Staugaard, 2009). Reaction time lacks the sensitivity required to

discriminate between covert attentional processes that underlie attentional bias (Cisler & Koster, 2010). Research conducted by Kappenman, Farren, Luck and Proudfit (2014) suggests that experiments using event related potentials (ERPs) in conjunction with dot-probe tasks, as measures of attentional bias, may produce more robust results. ERPs are able to measure cortical brain activity at millisecond precision to further disentangle the underlying attentional processes (Pfabigan, Lamplmayr-Kragl, Pintzinger, Sailer & Tran, 2014).

Event Related Potentials (ERPs): Indices of Attentional Bias

Event-related potentials (ERPs) are a high temporal resolution measure of the time course of cortical brain activation associated with perceptual and cognitive processes (Hillyard & Anllo, 1998). They are able to discriminate early pre-conscious attentional processes from conscious allocation of attentional resources (Bar Haim, Lamy & Glickman, 2005; Mangun, 1995). Using encephalography (EEG), ERPs comprise of averaged recordings of electrophysiological cortical activity time-locked in response to a stimuli. A typical waveform is derived from the averaged EEG to a stimulus, and has characteristic positive and negative ongoing waveforms which are defined by time post stimulus onset. Some early ERP components, such as P1 and N1 (prior to 200ms post-stimulus onset), are thought to reflect early, automatic attentional processes (Hillyard & Anllo-Vento, 1998; Bar Haim, et al., 2005; Näätänen, 1992). Later ERP components, such as P3, are regarded as reflecting conscious attentional processing of salient stimuli (Hajcak, MacNamara, & Olvet, 2010). It is well established that as greater attention is allocated to a stimuli, a higher level of cortical activation occurs, resulting in larger amplitudes in ERP recordings (Vogel, Woodman, & Luck,

2000). Recent research has started to combine ERP measures with reaction time to assess attention to threat in dot probe tasks, allowing for the observation of the time course of attentional bias with millisecond accuracy (Kappenman, et al., 2014). Three components all implicated in visual attention and its respective allocation to stimuli are P1, N1 and P3 components**.**

P1 Component: The P1 component is the first positive peak after stimuli onset, reflecting extrastriate visual cortex activation, characteristically at occipital sites. P1 amplitudes are maximal at approximately 80ms to 130ms post stimulus onset (Mangun, 1995; Sass, Heller, Stewart, Silton, Edgar, Fisher et al., 2010). Typically, this is a reflection of unconscious early allocation of cognitive resources to visual stimuli within the environment and can be observed over parietal, frontal and occipital sites (Hillyard & Anllo-Vento, 1998; Bar Haim, Lamy & Glickman, 2005). In response to the simultaneous presentation of neutral and fearful emotional faces, Pourtois, Grandjean, Sander, and Vuillemier (2004) found that P1 components were larger in response to fearful faces. Additionally, Sass, et al., (2010) demonstrated gender differences in time taken to process threatening stimuli between men and women on an emotion-word Stroop task, another behavioural task measuring attentional bias. They found women had greater attentional bias toward threat indicated by greater P1 amplitudes. Heightened P1 components have also been implicated in the early processing of threat words measured during a Stroop task (Li, Zinbarg & Paller, 2007).

N1 Component: The N1 component is characterised by a large negative amplitude peak at approximately 50 to 150ms latency following stimuli onset, which is implicated as evidence of early unconscious attentional processing reflecting arousal

(Näätänen, 1992; Lithari et al., 2010; Liu et al., 2012). N1 components are maximal at fronto-central midline topographical sites (Schupp, Flaisch, Stockburger, & Junghöfer, 2006). Lithari et al. (2010) revealed that in response to passive viewing of images from the International Affective Picture System (IAPS; Lang et al., 1997), females had enhanced N1 components compared to males, especially to unpleasant images. This effect was modulated by autonomic arousal, with high arousal evoking greater ERP amplitudes in both females and males (Lithari et al., 2010). Furthermore, Gardner, Carr, MacGregor and Felmingham's (2013) revealed that females had increased automatic attentional processing to negative IAPS images, as measured by greater N1 amplitudes. Whilst sparse, current ERP studies reveal that females display larger early P1 and N1 amplitudes to threat, which may reflect an automatic attentional bias towards threat. However, this needs to be assessed in combination with a dot-probe task.

P3 Component: The P3 component is a large positive peak occurring approximately 300ms from stimuli onset and is predominately observed over centro-parietal sites (Hajcak, et al., 2010). P3 components reflect the later allocation of conscious attentional and processing resources to salient, and task-relevant stimuli (Polich, 2007). Thomas, Johnstone, & Gonsalvez (2007) revealed greater P3 amplitude for unpleasant words, compared to neutral words, in an emotion-word Stroop task, which is indicative of preferential processing of unpleasant words. Additionally, sex differences in P3 amplitudes have also been observed. Females demonstrated greater P3 amplitudes, compared to males, when presented with relevant versus irrelevant visual stimuli (Steffenson et al., 2008). In a global-local paradigm, females revealed enhanced P3 amplitudes to local targets, suggesting greater resource allocation in the visual

processing of these stimuli types (Roalf, Lowery & Turetsky, 2005). Finally, on analysis of P3 amplitude peak, Kappenman, MacNamara & Proudfit (2014) found that despite no behavioural attentional bias being detected during dot-probe tasks, as measured by reaction time, ERP results indicated an instantaneous movement of visual attention to threatening IAPS images. It was concluded that reaction time was not sensitive enough to detect any covert attentional processes but ERP measures were.

Referring back to Bar Haim and colleague's (2007) Model of Attentional Bias, early unconscious allocation of attentional resources to label a stimuli as threat or non-threat (the PTES) and initial orientation to threat (RAS) can be associated with early P1 and N1 components as they reflect the early orientation of attention to salient stimuli. Additionally, the GTES is the more conscious allocation of our attentional processes, using resources such as memory to evaluate the threat. This later stage can be related to the P3 component, a later positive peak of neuronal activity indicative of later, conscious visual attention.

In summary, increased arousal and attentional bias towards threat may be a potential mechanism of female vulnerability for anxiety disorders (Cisler & Koster, 2010; Gorman, Kent, Sullican & Coplan, 2000). Despite the knowledge that females have twice the life time prevalence of anxiety disorders compared to males (Kessler, Chia, Emler, Merikangas & Walters, 2005)**,** there is currently a gap in the research examining sex differences in attentional bias and arousal. Research has yet to combine the dot-probe paradigm with a pre- and post- stress induction biomarker measure whilst recording brain wave activity, which enables the delineation of automatic and conscious

attentional processing, and offers the potential for a more precise examination of component processes in attentional biases.

Aims and Hypotheses

The aim of the current study is to replicate and extend the findings of Carr, Scully, Webb & Felmingham (2015) by examining sex differences in arousal and attentional bias before and after an acute stress induction task, whilst adding ERP measures of attentional bias to threat alongside reaction time measures and salivary measures of arousal (salivary alpha amylase reflecting noradrenaline). It is hypothesised that;

H^1: Female participants will display greater arousal reactivity to acute stress induction (the Cold Pressor Stress task) compared to males, measured by increased levels of noradrenaline (salivary alpha amylase).

H^2: Female participants will show attentional avoidance, rather than bias, to threat in the dot-probe task at baseline compared to males, as measured by slower reaction time and lower event related potential amplitudes (P1, N1 and P3), to congruent trials compared to neutral trials.

$H^{3:}$ Following the acute stress induction (the Cold Pressor Stress task) female participants will display a significantly greater attentional bias towards threat compared to males, reflected in faster RT and higher ERP amplitudes (P1, N1, P3) to congruent vs neutral trials compared to males.

Method

Design

The present study utilized a 2 (Sex: Male/Female) x 2 (Stress: Pre/Post CPS) mixed factorial design with Sex as the between factor and Stress as the within factor which was employed to measure physiological arousal pre and post stress induction. The dependent variable was salivary alpha amylase, reflecting endogenous noradrenaline levels. A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Neutral-Neutral/Incongruent) x 2 (Stress: Pre/Post CPS) mixed factorial design with Sex as the between factor, and Condition and Stress as within factors. The dependent variables were the peak amplitude for the P1, N1 and P3 (Site was an additional factor for the ERP analyses) components and averaged reaction time during the dot-probe task.

Participants

Nineteen males and 18 female participants (n = 37, M_{age} = 25.24 years, SD = 6.24) years) made up the sample. Participants were recruited from the University of Tasmania and comprised partially of first year psychology students, who received two hours course credit for their participation. The rest of the sample was made up of individuals from the community recruited through word-of-mouth snowballing. Participants were excluded if they had a history of psychiatric illness, neurological disorders including epilepsy, pregnancy (due to salivary measures), and substance abuse or had any type of traumatic brain injury. This was determined by responses on a Participant Demographic and Clinical Screening Questionnaire (see Appendix A). To control for cognitive decline, participants were under the age of 55. Ethics approval was obtained from the Social Sciences Human Research Ethics Committee (Appendix B).

Apparatus/Instrumentation/Materials

Depression, Anxiety and Stress Scales (DASS - 21)

To assess affect on the day, the 21-item Depression, Anxiety and Stress Scales (DASS) (Lovibond & Lovibond, 1995) was administered (Appendix C). The DASS is a self-report questionnaire measuring levels of depressed, anxious and stressed mood over the past week on a four-point Likert scale. Scores calculated from the DASS were used to indicate an estimation of variability in participants' mood states. The DASS has been shown to be a highly reliable indices of depression, anxiety and stress (Cronbach's alpha of .95, .90 and .93 respectively).

Traumatic Event Questionnaire (TEQ)

To categorise participants as either trauma exposed (TE) or non-trauma exposed (NTE), the Traumatic Event Questionnaire was completed (see Appendix D). The TEQ is an 11 item questionnaire, assessing nine life events such as the experience of sexual abuse, news of the serious injury or death of someone, serious accident, as well as being able to examine unspecified traumatic events (Vrana & Lauterbach, 1994). The TEQ was administered as a measure embedded in another study using the same sample.

Post-Traumatic Stress Disorder Checklist (PCL-5)

Supplementary to the TEQ, the 20-item Post-Traumatic Stress Disorder Check (PCL-5; Weathers, Litz, Herman, Huska & Keane, 1993; see Appendix E) was administered. The PCL-5 is a self-report measure that assesses the 20 DSM-5 symptoms of PTSD. The PCL-5 was administered as a measure embedded in another study using the same participant sample.

Saliva samples

Salivary measures of noradrenaline (NA) were obtained using sAA. Saliva samples were collected at baseline and post-acute stress induction in standardized saliva collection tubes using the passive drool method (naturally induced). sAA is a reliable biological marker of noradrenaline which has been shown to reflect sympathetic arousal (Rohleder & Nater, 2009). Saliva samples were immediately frozen at -20°C. Samples were analysed with commercially available kits (Salimetrics, USA) at Macquarie University. Thawed samples were centrifuged at 1500 x g for 15 min and all samples were analysed in duplicate. Thawed and centrifuged sAA saliva samples were diluted 1:200.

Dot-probe task:

A dot-probe paradigm was used to measure behavioural (reaction time) and ERP responses to threat stimuli. Stimuli for this task were a series of negative and neutral images selected from the standardized and widely used International Affective Picture System based on normative data of arousal and valence ratings (IAPS; Lang et al., 1997). The valence for negative and neutral images were 2.92(.92) and 5.62(.98) respectively. Mean arousal of the negative and neutral images were 5.86(.82) and 3.67(.89). One-hundred and fifty-two neutral images and 75 threatening images were selected. Images were randomly paired into neutral/neutral (for the neural condition) or threat/neutral combinations (for the congruent and incongruent conditions). There were three conditions; congruent, neutral-neutral and incongruent. Each block of the dot-probe task comprised of 56 trials, with different images appearing in each block.

Given that the current study was attempting to replicate the behavioural study of Carr et al., (2015), the paired stimuli were presented for 1000ms after onset.

Immediately after this a probe appeared behind the target image; it's location dependent on condition (behind the threatening image in the congruent condition, behind the neutral image in the incongruent condition, and behind one of the neutral images that were simultaneously presented in the neutral condition). The probe appeared for 400ms, before a 2000ms response period. Participants were required to indicate whether the probe appeared on the left or right on a keyboard ('A' key or 'L' key respectively). In congruent trials, the probe would appear behind the threat image in a neutral/threat pairing. For incongruent trials, the probe would present behind the neutral image in a neutral/threat paring. In neutral trials, the probe would appear behind either image in neutral/neutral pairing.

Attentional bias to threat is evident by faster reaction times on congruent trials compared to incongruent conditions. As such, attentional bias in a dot-probe task can be inferred by faster reaction time in congruent trials, suggesting attentional engagement or facilitated attention to threat, and by slower response times in incongruent trials indicating slow disengagement away from threat (Van Bockstaele et al., 2014; Koster, Crombez, Verschuere, & De Houwer, 2004). Attentional avoidance is defined by slower reaction time in congruent trials compared to incongruent trials which is attributed to the predisposition to shift attention away from threatening stimuli (Carr et al., 2015; Sagliana, Trojana, Amoriello, Miglozzi & D'Olympio, 2014).

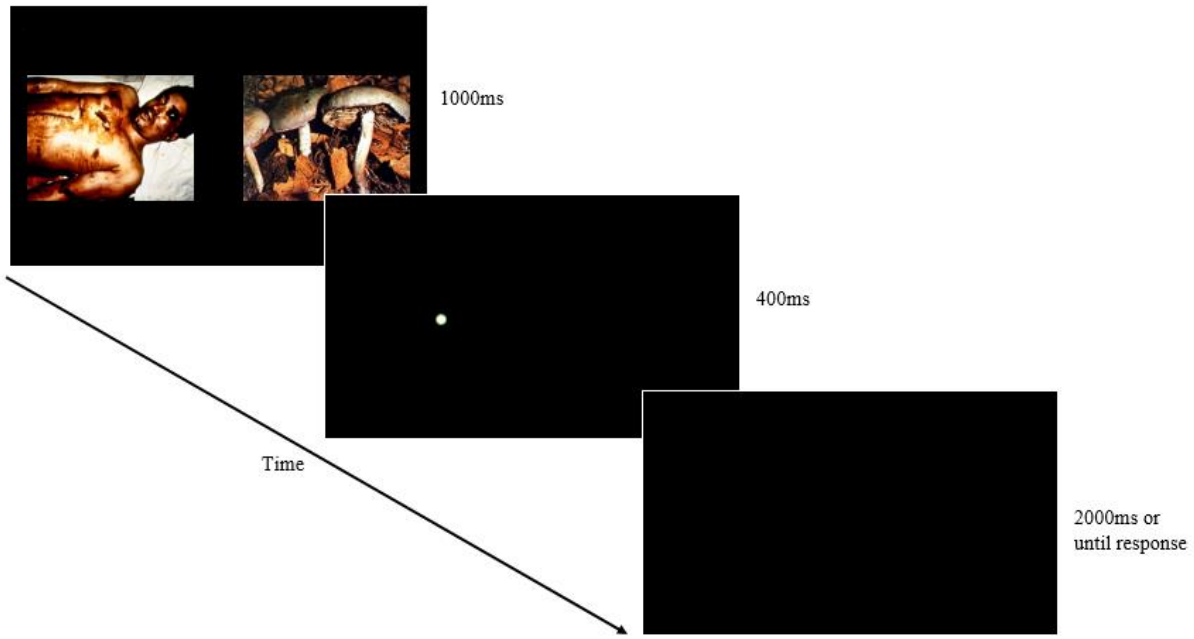

Figure 2. Example of congruent dot-probe trial.

***Electrophysiological (EEG) recording*:**

NeuroScan[2] and STIM software was used to program and administer the dot-probe task on a Celeron D Class computer. EEG data was recorded continuously using a NeuroScan SynAmps[2] system, SCAN 5.2 software and a 32 channel Quick-cap with silver and silver-chloride (Ag/AgCl) electrodes. EEG recordings were recorded from 32 sites and positioning of the electrodes was in accordance with the International 10-20 system (Jasper, 1958). The reference points for the electrodes were the linked mastoids and AFz ground. Electrode impedance was maintained at or below 5kΩ throughout all tasks. Data was corrected for ocular artefact via vertical electroculogram (VEOG) electrodes attached to the supra- and infra orbital sites of the left eye and horizontal electroculogram (HEOG) electrodes positioned on the outer canthi of both eyes.

Reflecting the typical maximal topographies of ERP components, ERPs were used to measure the cortical activity associated with automatic visual attention at parietal cortex (P1 component), automatic visual attention at frontal sites (N1 component) and later, conscious allocation of visual attention (P3 component) at parietal sites. Attentional bias in ERP measures will be inferred by enhanced ERP amplitudes in congruent trials compared to incongruent and neutral. Attentional avoidance is indexed by decreased ERP amplitude in congruent trials compared to incongruent and neutral trials.

Cold Pressor Stress (CPS) Task

A standard procedure was employed for the CPS task which has been shown to reliably increase physiological stress reactions, such as increased noradrenaline levels in humans (Carr, et al, 2015; Van Stegeren Wolf & Kindt, 2008). A bucket of cold water maintained at, or below 4 degree Celsius was used to induce stress during the task. The CPS task has Participants were required to submerge their dominant hand up to the wrist in bucket of water for no longer than three minutes or until it became uncomfortable, but not painful. Time of immersion was also recorded for each participant.

Procedure

Each potential participant completed the Demographic and Clinical Screening Questionnaire (Appendix A) and this information was used to screen for exclusion criteria. After providing eligible participants with an information sheet (Appendix F) and obtaining informed consent (Appendix G), they were prepped for ERP recording, being fitted with the electrode mounted cap. A baseline saliva sample was then collected from each participant, using the passive drool method. Salivary noradrenaline levels

were analysed by standard assays of salivary alpha-amylase (sAA) at the Macquarie University.

Prior to the dot-probe task, brief instruction were presented to the participants regarding the protocol for the dot-probe task (see Appendix H). A practice trial of five blocks was completed by each participant to ensure the understanding of experimental instructions. Participants then completed the first dot-probe task which was presented on a computer screen. Participants sat at a standard 50 cm away from the screen. Following this, participants completed the CPS task. Immediately after the CPS task a second saliva sample was collected as a measure of post-stress induction NA levels. Finally, the second dot-probe task was administered

To ensure that the participants did not deviate from the norms of arousal and valence of the IAPS images, a picture rating scale was administered. Participants had to rate on a scale of one to ten their perceived valence and how arousing they found each image, which was then averaged and compared between threat and neutral images for females and males.

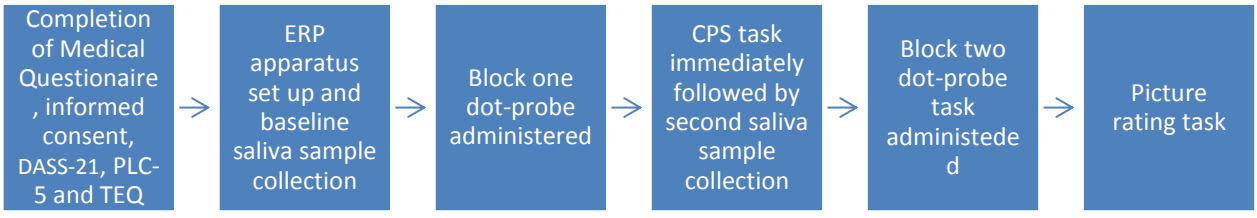

Figure 3. Flow chart of procedure.

Analysis

Reaction time was averaged for each trial type (Congruent, Incongruent and Neutral/Neutral). ERP measures were calculated according to standard analysis definitions and procedures. Maximal P1 amplitudes were defined at parietal sites (Pz, P3 and P4). N1 maximal amplitudes were observed at fronto-central and central sites (FCz and Cz) and parietal, centro-parietal and central sites. The greatest P3 amplitudes were found at centro-parietal, central, and parietal mid-line sites (CPz, Cz and Pz). Therefore the analysis of P1, N1 and P3 were restricted to their respective topographical sites (Hillyard & Anllo-Vento, 1998; Bar Haim, Lamy & Glickman, 2005; Schupp, Flaisch, Stockburger, & Junghöfer, 2006; Hajack et al., 2010). Peak selection and mean amplitude windows were guided by grand mean averages and supported by previous literature; P1 component from 80 to 150ms post stimulus onset (Mangun, 1995; Sass, Heller, Stewart, Silton, Edgar, Fisher et al., 2010), N1 component from 50 to 150ms latency following stimuli onset (Näätänen, 1992), and P3 component at approximately 300ms from stimuli onset (Hajack, et al., 2010).

Univariate ANOVAS were used to analysis demographic and clinical data including age, depressed mood, anxiety, stress (measured by the DASS-21) and trauma exposure (measured by PCL-5 total). To analysis saliva samples, a separate 2 (Sex: Male/Female) x 2 (Time: Pre/Post stress induction) mixed factor ANOVA was employed. A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Neutral-Neutral/Incongruent) x 2 (Stress: Pre/Post CPS) mixed design ANOVA was run to analyse mean reaction time across all conditions. A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Neutral-Neutral/Incongruent) x 2 (Stress: Pre/Post CPS) x 3

(Site: P3/P4/Pz/FCz/CPz/Cz)[2], mixed design ANOVA was run to analyse ERP components. Significance was set at $p < 0.05$ and effects size and confidence intervals were examined for all analyses. Sidak post-hoc tests were used to break down analysis when required. To remove the consideration of sphericity, multivariate analysis results and Wilk's Lambda were reported (Field, 2013). Chi-square analysis was utilized to observe the distribution of attentional bias and avoidance, and trauma exposed and non-trauma exposed across females and males. Finally, a bivariate correlation was used to examine the relationship between sAA and reaction from pre- to post-stress.

Results

Demographic and Clinical Data

Separate univariate ANOVAs were conducted to determine if there were any sex differences in age, depressed mood, anxiety and stress (as measured by the DASS; Lovibond & Lovibond, 1995). A univariate ANOVA was run to determine sex differences in PTSD symptom severity between males and females (as measured by the PCL-5). No significant differences were found for age, depressed mood, anxiety, and stress, or PCL-5 total (See Table 1). Chi-square analysis was conducted to explore Sex differences of trauma exposure (classified using the TEQ). It revealed no significant difference between the number of females and males in the trauma exposed (TE) and the non-trauma exposed (NTE) groups (summarized in Table 1).

[2] Specific sites were selected for each component according to their maximal topography, as outline above.

Table 1. Mean Scores, Total Statistics (F & χ^2) , probability values and effect sizes for Age, Depressed Mood, Anxiety, Stress, PCL total score, and TE and NTE for Males and Females.

Variable	Males	Female	*Total statistic*	*p*	η_p^2
Age	25.26(5.68)	25.22(6.95)	*F* =0.00	.98	.00
Depressed mood	2.00(2.45)	2.89(4.69)	*F* =.53	.47	.02
Anxiety	1.53(1.71)	2.78(3.25)	*F* =2.19	.15	.06
Stress	3.37(2.56)	4.50(4.48)	*F* =.90	.35	.03
PCL-5 total	8.32(6.06)	11.78(12.85)	*F* =1.12	.30	.03
TE or NTE	11 (TE)	7 (TE)	χ^2 = 1.34	.33	-
	8 (NTE)	11 (NTE)			

Salivary Alpha Amylase Data

Mean sAA levels, measured in units per volume (U/mL), from pre-stress baseline to post-stress time points for males and females are summarised in Figure 4. A 2 (Sex: Male/Female) x 2 (Time: Pre-/Post-stress) univariate ANOVA was conducted revealing a significant main effect of Sex ($F_{(1,35)}$ = 1.81, p = .049, η_p^2 = 0.50) whereby males demonstrated higher sAA levels overall, compared to females. There was also a significant main effect of Time ($F_{(1,35)}$ = 4.71, p = .047, η_p^2 = 0.12, λ = .88) by which noradrenaline levels significantly increased post-stress induction compared to pre-stress levels. However, no significant interaction of Sex and Time was evident ($F_{(1,35)}$ = .49, p = .49, η_p^2 = 0.01, λ = .99).

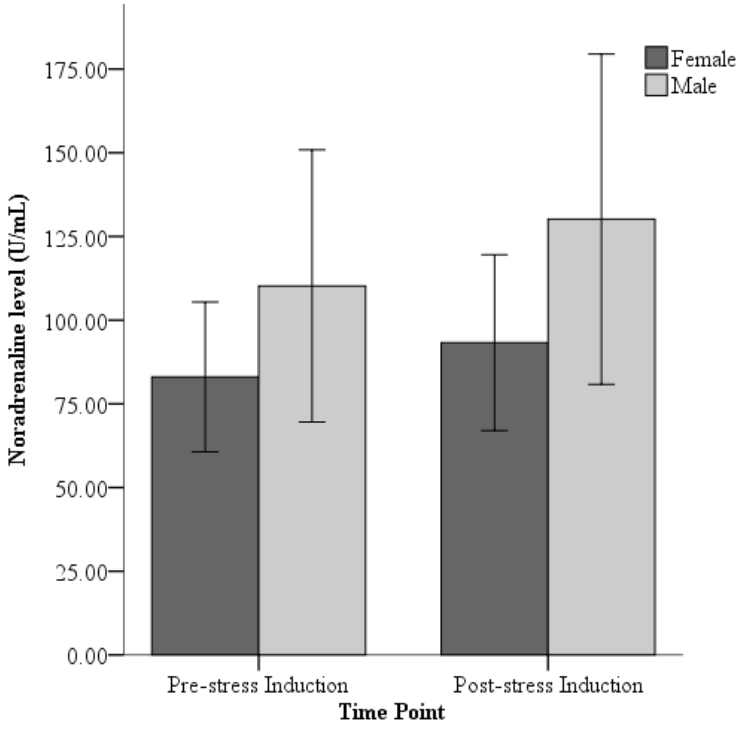

Figure 4. Mean noradrenaline level (U/mL) pre- and post-stress induction for Males and Females including error bars (95% confident intervals).

Reaction Time Analysis

Reaction time (RT) in milliseconds was scanned for missing values and outliers. Individual scores that were more than three standard deviations from the mean score for pre- and post-stress for each condition, congruent, incongruent and neutral, were replaced with values lying just inside the three standard deviation threshold (Tabachnick & Fidell, 2007). No outliers for males or females were observed.

Means and standard deviations were calculated for RT during the dot-probe task (See Table 2). A 2 (Sex: Female/Male) x 3 (Condition: Congruent/Incongruent/Neutral) x 2 (Time: Pre-/Post-stress) repeated measures ANOVA revealed significant main effect of Sex ($F_{(1, 35)} = 5.37$, $p = .03$, $\eta_p^2 = .13$) whereby RTs across all conditions were faster for males compared to females (refer to Figure 5). A significant main effect of Time

was observed ($F_{(1, 35)} = 36.99$, $p < .001$, $\eta_p^2 = .51$, $\lambda = .49$), with overall RT significantly faster at post-stress induction compared to pre-stress induction RT (see Figure 5). No main effect of Condition was noted and no significant interactions between Sex, Condition, and Time were evident (for details of non-significant effects, see Table 2 in Appendix I).

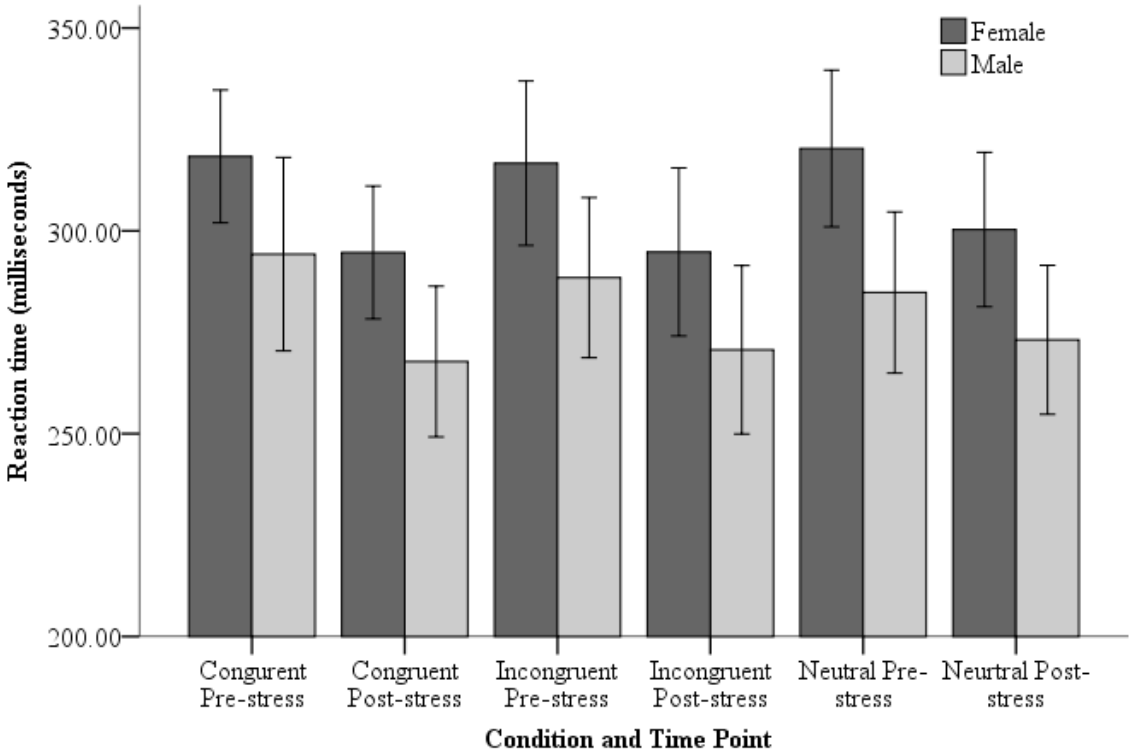

Figure 5. Mean reaction times for condition at pre- and post-stress induction time points for Males and Females. Error bars indicate 95% confidence intervals using standard error.

Event Related Potential Data

ERP amplitude in microvolts (μV) data was screened for missing values and outliers. Individual scores that were greater than three standard deviations above or below the mean score (5.85% in females and 1.35% in males) for pre- and post-stress

for each condition; congruent, incongruent and neutral, were substituted with values just inside the three standard deviations range from the mean of the corresponding sex group and condition for each ERP component at the relevant site (Tabachnick & Fidell, 2007)[3].

Grand Mean Averages

Grand mean average waveform examples for males and females across congruent, incongruent and neutral conditions, pre- and post-stress inductions are depicted in Figures 6a, 6b and 6c. As observed in Figures 6a, 6b and 6c, peak selection and mean amplitude windows were determined by grand mean averages and supported by previous research, as previously discussed.

[3] Site effects not specifically relevant to hypotheses are not reported in text, but can be found in the appendices.

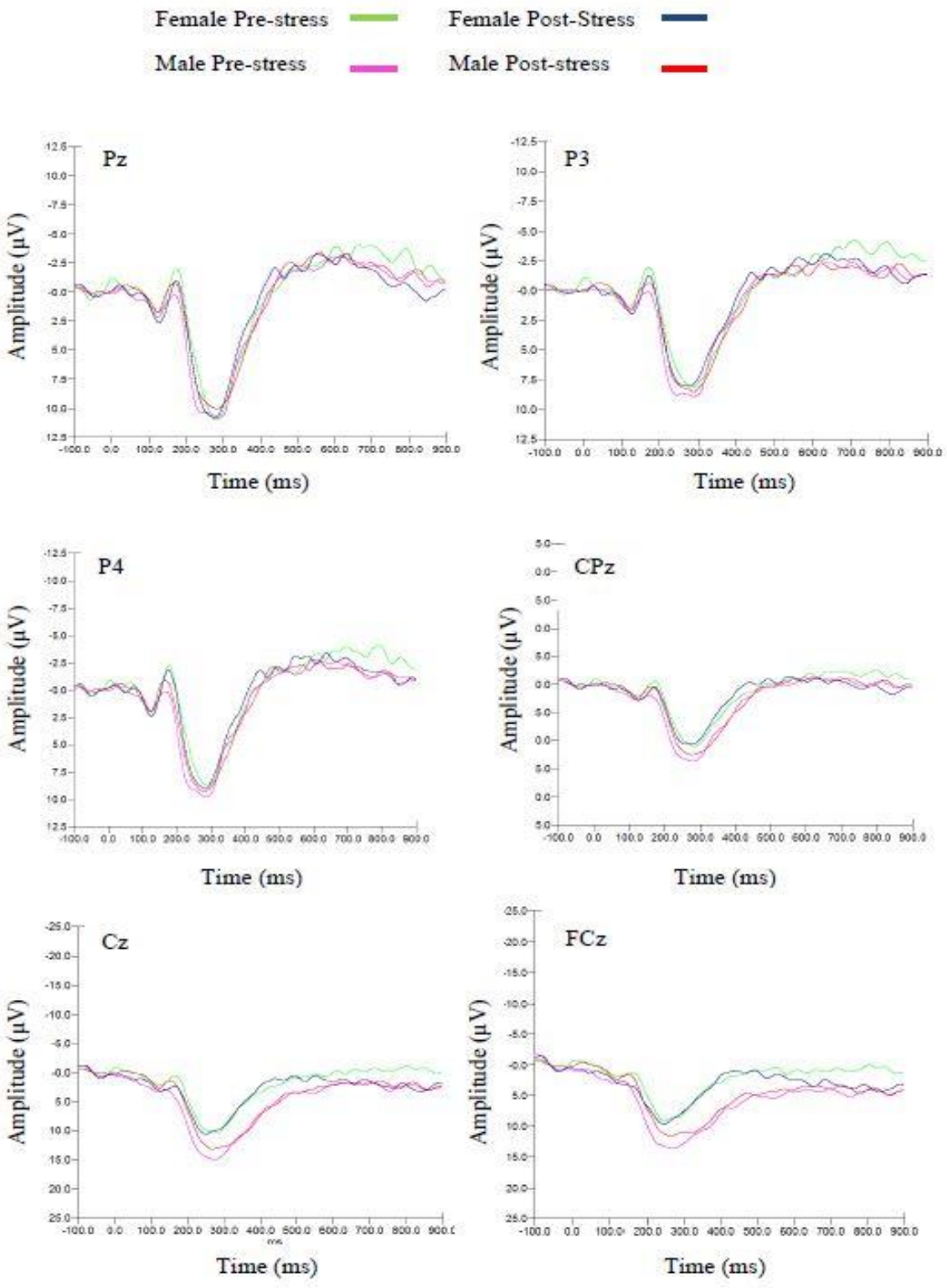

Figure 6a. Grand mean average waveform for females and males in congruent condition, pre- and post-stress, at sites Pz, P3, P4, FCz, Cz and CPz.

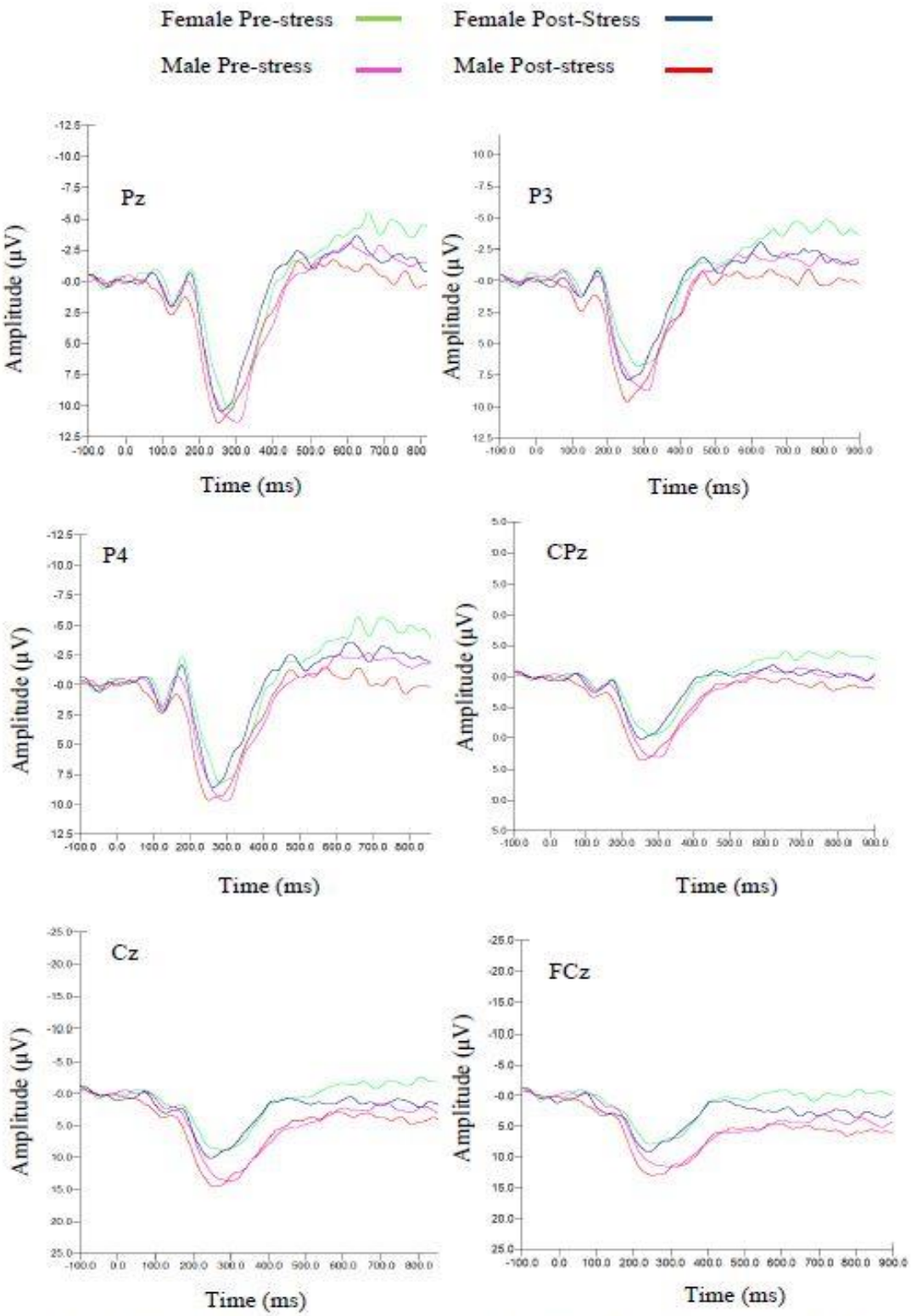

Figure 6b. Grand mean average waveform for females and males in incongruent condition, pre- and post-stress, at sites Pz, P3, P4, FCz, Cz and CPz.

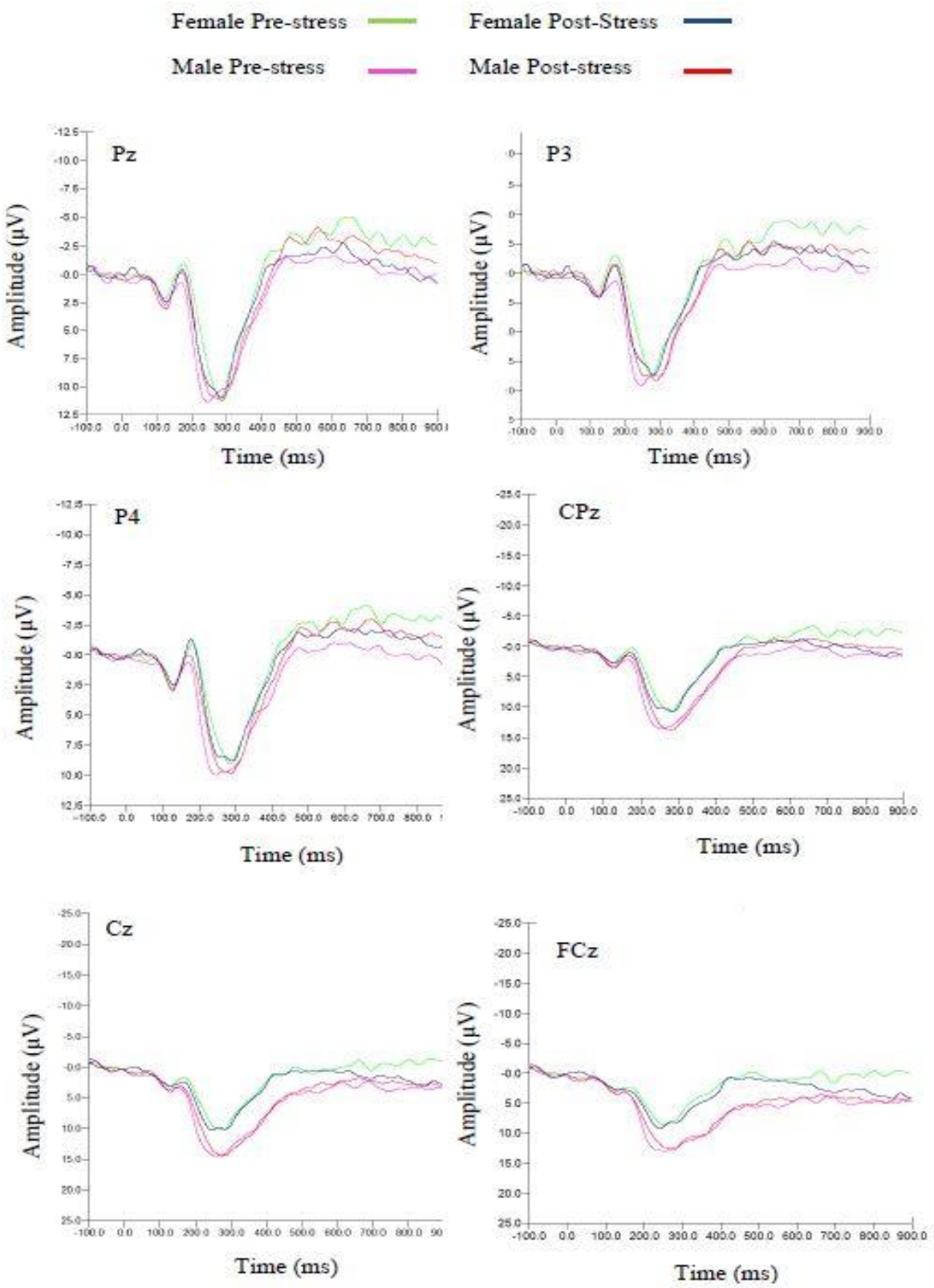

Figure 6c. Grand mean average waveform for females and males in neutral condition, pre- and post-stress, at sites Pz, P3, P4, FCz, Cz and CPz.

P1 Amplitude

A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Incongruent/Neutral) x 2 (Time: Pre-/Post-stress) x 2 (Site: Pz/P3/P4) repeated measures ANOVA was conducted. No main effect of Sex or Time were revealed, these results are summarised in table 3. Significant main effects of Site ($F_{(2, 34)} = 5.33$, $p = .01$, $\eta_p^2 = .24$, $\lambda = .76$) and Condition were revealed ($F_{(2, 34)} = 3.88$, $p = .03$, $\eta_p^2 = .19$, $\lambda = .81$). Sidak-adjusted pairwise comparisons of the Site main effect indicated a significant difference in P1 amplitude across sites; P1 amplitude were significantly greater at Pz ($M = 3.39$, $SE = .47$) compared to P4 ($M = 2.73$, $SE = .36$; $p = .01$, 95% CIs [.14, 1.18]). No significant differences were revealed between Pz and P3 ($p = .29$, 95% CIs [-.13, .66]) or P3 and P4 ($p = .22$, 95% CIs [-.15, 95]). No significant interaction were observed between Sex, Time, Condition or Site, these results are summarized in Table 3 in Appendix J.

Pairwise Sidak-adjusted comparisons for the Condition main effect (summarised in Figure 7) revealed a significant difference in P1 amplitude between incongruent and neutral conditions, by which the neutral condition elicited a greater P1 amplitude compared to the incongruent condition ($p = .02$, 95% CIs [-1.04, -.08]). There were no significant difference between Congruent and Incongruent conditions ($p = .96$, 95% CI [-.50, .70]) or Congruent and Neutral conditions ($p = .25$, 95% CI [-1.11, .20).

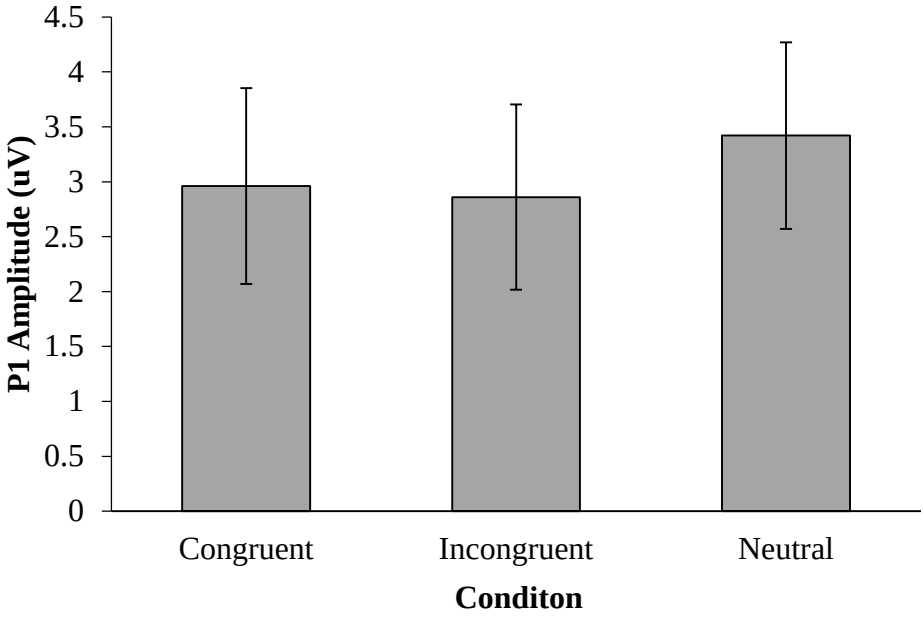

Figure 7. Main effect of Condition for P1 amplitude. Error bars indicate 95% confidence intervals using standard error.

N1 Amplitude

A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Incongruent/Neutral) x 2 (Time: Pre-/Post-stress) x 2 (Site: FCz/Cz) repeated measures ANOVA was conducted which revealed no significant main effect of Sex ($F_{(1, 35)}$ = .43, p = .52, η_p^2 = .01), Condition ($F_{(2, 34)}$ = .1.46, p = .25, η_p^2 = .08, λ =) or Site ($F_{(1, 35)}$ = .11, p = .74, η_p^2 = .00, λ =). A significant main effect of Time was observed ($F_{(1, 35)}$ = 4.75, p = 0.04.46, η_p^2 = .12, λ =), by which N1 amplitude was significantly greater pre-stress induction (M = -.28, SE = .49), compared to post-stress induction (M = .74, SE = .66) time point. No significant main effects of Sex was found and no significant interactions between Sex, Condition, Time, and Site were revealed. These non-significant results are summarised in Table 4 in Appendix K.

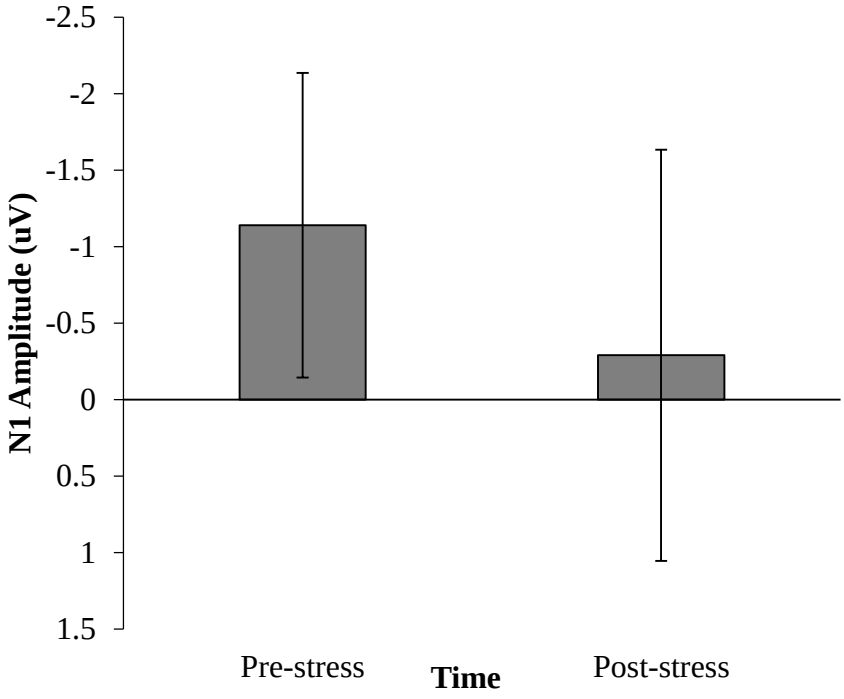

Figure 8. Main effect of Time for N1 amplitude. Error bars indicate 95% confidence intervals using standard error.

P3 Amplitude

A 2 (Sex: Male/Female) x 3 (Condition: Congruent/Incongruent/Neutral) x 2 (Time: Pre-/Post-stress) x 3 (Site: CPz/Cz/Pz) repeated measures ANOVA was conducted. No significant main effects of Sex, Condition or Time were discovered with results summarised in Table 5, Appendix L. A significant main effect of Site was revealed, ($F_{(1,35)}$ = 6.04, p = .006, η_p^2 = .26, λ = .74). Sidak-adjusted pairwise comparisons indicated a significant difference between Cz and CPz (p = .004, 95% CIs [-1.88, -.31]) with P3 amplitude being greater than at Cz (refer to Figure 9). Further, a significant Site difference was observed between Cz and Pz (p = .006, 95% CIs [-2.91, -.41]; refer to Table 9)

A significant interaction was observed between Site and Sex ($F_{(2, 34)} = 6.06$, $p = .006$, $\eta_p^2 = .26$, $\lambda = .74$). Sidak adjusted pair-wise comparison analysis indicated a trend at Pz for females to have a lower P3 amplitude at Pz compared to males ($p = 0.57$, 95% CIs [-7.42, .12]). No other significant differences were found between females and males at Cz ($p = .78$, 95% CI [-.3.48, 2.64]) or CPz ($p = .13$, 95% CI [-6.10, .82]).

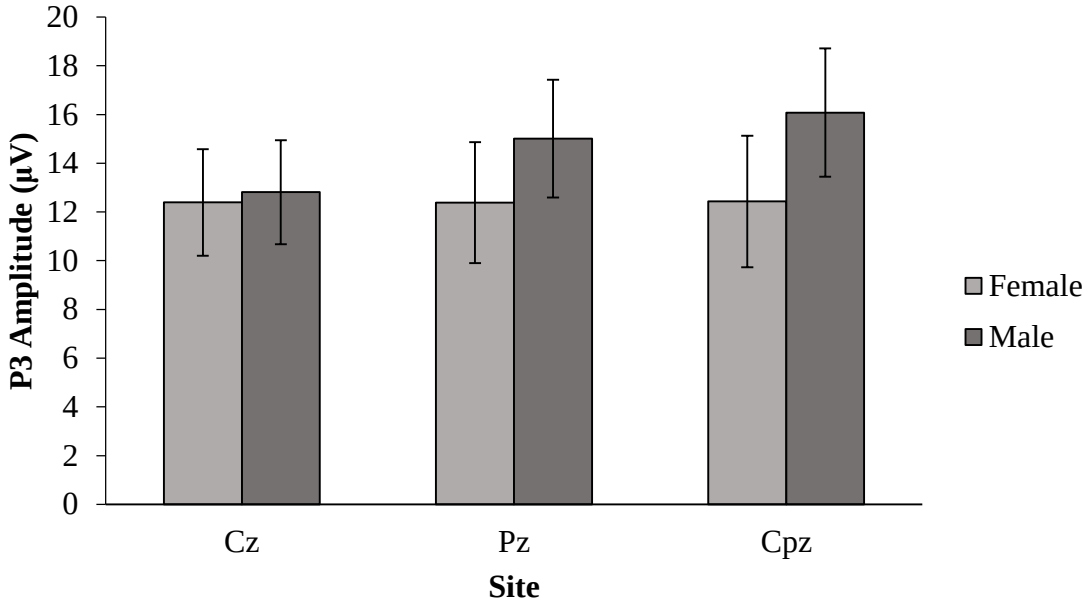

Figure 9. Interaction of Site x Sex for P3 amplitude. Error bars indicate 95% confidence intervals using standard error.

Valence and Arousal Ratings

Means and standard deviations for valence and arousal of threat and neutral images were calculated from responses on the picture rating task, and are summarised in Table 6.

Table 6. Means and standard deviations (in parenthesis) for picture rating of IAPS images used in dot-probe paradigm.

Dependent Variable	Threat Image	Neutral Image
Valence	4.84(.70)	4.74(.69)
Arousal	3.06(1.04)	3.00(1.13)

To analysis arousal ratings, a 2 (Image: Threat/Neutral) x 2(Sex: Female/Male) univariate ANOVA found there was no significant main effect of Image ($F_{(1,28)}$ = .63, p = 0.44, η_p^2 = .02, λ = 1.00). There was also no significant main effect of Sex on arousal picture ratings ($F_{(1,28)}$ = 2.99, p = 0.10, η_p^2 = .10). No significant interaction between Sex and Image was revealed ($F_{(1,28)}$ = .02, p = 0.90, η_p^2 = .00, λ = 1.00).

To examine valence ratings, a 2 (Image: Threat/Neutral) x 2(Sex: Female/Male) univariate ANOVA revealed no significant main effect of Image ($F_{(1,28)}$ = 2.99, p = 0.10, η_p^2 = .10, λ = .90). There was also no main effect of Sex on valence ratings ($F_{(1,28)}$ = 1.30, p = 0.26, η_p^2 = .04). No significant interaction between Sex and Image was revealed ($F_{(1,28)}$ = .08, p = .79, η_p^2 = .00, λ = 1.00).

Control Analysis

Given the surprising lack of evidence for an attentional bias in either RT or ERP measures, the pattern of responses in each individual participant for female and males was examined. Using RT as an index, a chi-square was conducted to investigate Sex differences for the tendency for attentional avoidance (RT slower at base line in congruent trials compared to incongruent trials) or attentional bias (RT faster at base line in congruent trials compared to incongruent trials). Chi-square analysis indicated that there was no significant difference between the number of females and males in the

attentional bias and the attentional avoidance group, with approximately half the females and the half the males adopting each strategy, which is summarised in Table 7.

Table 7. Chi-square analysis of attentional bias or attentional avoidance.

Variable	Males	Female	χ^2	*p*
Attentional Bias (AB) or Attentional Avoidance (AA)	10 (AB) 9 (AA)	8 (AB) 10 (AA)	.25	.62

Correlation Analysis of sAA and Reaction Time Data

A bivariate correlation between baseline post-stress sAA and RT measures of attentional bias, assessed with Pearson's Correlation Coefficients, revealed no significant relationship, (r = -.08, p = .64).

Discussion

The aim of the present study was to examine sex differences in attentional bias during a dot-probe following an acute stress task using both reaction time and ERP measures. The results of the present study did not confirm the hypotheses of greater arousal and attentional bias in females following acute stress. The results indicate that there is a sex difference in noradrenaline level by which males have significantly higher levels of noradrenaline, as measured by sAA, compared to females. Additionally, noradrenaline levels were significantly greater in response to acute stress compared to pre-acute stress noradrenaline levels. Reaction time during the dot-probe task was faster for males across all conditions, compared to females. Furthermore, reaction time during the second dot-probe task was significantly faster following the acute stress induction compared to the initial dot-probe task. An increase in N1 amplitudes post the acute

stressor was observed. Females, compared to males, had lower P3 amplitudes at topographical site Cz.

Sex Differences in Noradrenaline to Stress

Noradrenaline levels (indexed by sAA) were found to significantly increase after acute stress induction from baseline, for both males and females. This finding confirms that the acute stress induction successfully increased stress and led to an increased release of the stress hormone noradrenaline. It was hypothesised that females would demonstrate greater arousal reactivity to an acute stress task, measured by increased noradrenaline levels, compared to males. Contradicting the hypothesis, males were found to have higher salivary alpha amylase levels (reflecting noradrenaline levels) overall, compared to females. The findings of this study were not in line with the results of Carr, Scully, Webb and Felmingham (2015), who found a significant interaction between stress and sex whereby males had greater baseline sAA levels than females, and females displayed significant increases in sAA from pre- to post-stress, where males did not. Current results did not support research such as Segal and Cahill's (2009) which indicated that noradrenaline responders to negative emotional images, indexed by sAA, were predominantly females.

However, other studies examining sex differences with salivary alpha amylase and the CPS task reveal findings consistent with the current study. Van Stegeren Wolf and Kindt (2008) found that exposure to either negative IAPs images or the CPS task both resulted in significant increases in sAA levels from baseline levels in females and males. Van Stegeren et al. (2008) reported males having higher sAA levels at baseline, which continued to be significantly higher than females across all conditions, which is

in accordance with the current study. Greater noradrenaline levels in males may have occurred due to heightened overall generalised hyperarousal. It may also be indicative of heightened anticipatory anxiety caused by knowledge of subsequent psychological testing which was also observed in Carr and colleague's results.

Another methodological difference between Carr et al. (2015) and the current study was the time point for collection of baseline saliva samples. In the current study baseline saliva samples were taken prior to exposure to any IAPS images, including those in practice trials, to reflect a true noradrenaline level. Comparatively, Carr et al (2015) collected a baseline saliva sample after participants had completed the first block of the dot-probe task, thus being exposed to negative facial expressions prior to saliva collection. sAA reactivity to stress induction has been shown to have a specific time course, with a rapid noradrenaline increase observed in approximately the first 20 minutes post-stress induction onset, before a decline is observed (Van Stegeren et al., 2008; Rohleder, Nater, Wolf, Ehlert & Kirschbaum, 2005). The difference in saliva collection time points in Carr et al (2015) and the present study may have led to their varying results. Additionally, exposure to negative imagery and the CPS task have been shown to elicit the same amount of autonomic arousal (indexed by increased noradrenaline levels; Van Stegeren et al., 2008), thus the baseline sAA levels in Carr and Collegues study may have been influenced by exposure to negative stimuli prior to baseline saliva collection.

Further explanation may come from the different stimuli used in the present study (IAPS images) compared to the emotional faces used in Carr and colleague's (2015). Despite there being limited research comparing both emotional stimuli,

functional magnetic resonance imaging (fMRI) research has found that there are overlaps in the brain regions activated by complex IAPS images and facial expressions (Britton, Taylor, Sudheimer & Liberzon, 2005; Aldhafeeri, Mackenzie, Kay, Alghamdi & Sluming, 2012) However, there are also distinctions (Britton, et al., 2005). Areas such as the superior temporal gyrus, insula, and anterior cingulate all uniquely activate when participants were exposed to emotional faces. These areas are typically associated with the processing of emotions and emotional arousal (Singer, Crichley & Preuschoff, 2009). Furthermore, it has been suggested that the amygdala preferentially processes facial expression (Britton, et al., 2005; Calder, Burton, Miller, Young & Akamatsu, 2001) and females have been shown to be more sensitive to facial expression, as indicated by increased ERP components, compared to males (Martinez & Du 2012; Orozco & Ehlers, 1998). Therefore, sex differences in the processing of emotional faces may have contributed to the findings of attentional bias in females' post-stress induction, which was not replicated in the current study, which used IAPS images.

Attentional Bias Effect in Reaction Time

The second hypothesis predicted that females would display attentional avoidance at baseline, measured by slower reaction times in congruent trials compared to incongruent and neutral trials, compared to males at baseline. Furthermore, it was predicted that females would demonstrate significant attentional bias after acute stress induction, as measured by faster reaction times in congruent trials compared to incongruent and neutral trials, compared to males from pre- to post-stress induction. Reaction time findings did not support these hypotheses.

There were no significant main effects of condition or time, and no significant interactions between sex and condition, or sex and time, or sex x condition x time. Thus, there was no evidence of avoidance in female participants at baseline, with reaction time not being significantly slower in congruent trials compared to incongruent and neutral trials in females. Furthermore, post-acute stress induction reaction times did not indicate attentional bias in any condition, and there were no significant sex differences. As such, these results did not support the findings of Carr et al., (2015) who found a significant avoidance effect at baseline and a significant attentional bias effect for females, compared to males. Overall, in the current study males had faster reaction times across all conditions compared to women, regardless of stress condition. This supports previous literature suggesting that males generally have quicker reaction times, compared to females, across several task paradigms (Carr et al., 2015; Der & Deary, 2006).

Unexpectedly there were no significant condition effects evident in reaction time data pre- or post- stress, suggesting that no attentional bias (or attentional avoidance) effects were found with the current task. Given the lack of condition effects in the data, and the lack of significant interactions between sex, condition and time, the present study cannot speak to changes to attentional bias pre- or post-stress. Attentional bias to threat in a dot-task is traditionally inferred by faster reaction time in congruent trials; whereby participants respond to a probe that appears behind a threatening stimulus, rather than neutral stimuli which is synonymous to facilitated attention (Cisler & Koster, 2010). It can also be extrapolated from slower reaction times on incongruent trials, which requires participants to respond to a probe appearing behind a neutral

image, which suggests difficulty disengaging from threat (Bar-Haim, et al., 2007; Cisler & Koster, 2010). Three potential explanations for not revealing any attentional bias effects in the current study may be related to stimuli duration time, the unreliability of reaction time as a dependent variable, and the variability of individual responses to the dot probe task (participants either displaying attentional bias or attentional avoidance within each group).

In the current study, a 1000ms stimuli duration was used to replicate the procedure used by Carr et al. (2015). Meta-analysis of dot-probe tasks attempting to produce attention bias suggests that the duration of stimuli presentation can have a profound effect (Bar Haim et al., 2007). Overall it was found that in non-anxious control samples there was no significant attentional bias effects with 500ms or $\geq$ 1000ms stimuli durations. It proposes that supraliminal, stimulus less than 500ms, stimuli were most effective in eliciting attentional bias (Bar Haim et al., 2007). Longer stimuli duration allows for more shifts of attention between stimuli before a response is required, which could explain why no definite attentional bias or avoidance was observed in the current study (Schechner et al., 2012). Consistent with these conclusions, Mogg, et al., (2004) found that stimuli duration of 1250ms and 1500ms in high-trait anxiety samples did not produce an attentional bias despite it being observed at 500ms duration. Using three different stimuli durations, Koster, Verschuere, Crombez and Van Damme (2005) found an attentional bias towards threat at 100ms and 500ms but any effect was absent at 1250ms. A further review of attentional bias literature using dot-probe paradigms was completed in the current study and results indicated that of 16 research papers using either IAPS images, emotional faces, or word pairings. All 15

experiments used stimuli durations of 500ms, with five including additional, varying stimuli durations. A summary of this review can be found in Table 6, Appendix M.

It is suggested that because of the stimuli duration time of 500ms often produces an attentional bias effect and stimuli durations greater than 1000ms elicits attentional avoidance the use of 1000ms stimuli duration in the current study may contribute to the lack of attentional bias effect. The stimuli duration may have been too long for attentional bias but too short for attentional avoidance. A more robust design would be to have multiple manipulations of stimuli duration times for the dot-probe task. This was not possible in the current study as it would mean the inclusion of a fifth independent variable thus the interpretation of a five-way interaction, which was outside the scope of the honours project.

The long stimulus duration times may have led to variability in the strategy chosen amongst individual participants (whether they demonstrated attentional biases to threat, or avoidance). Comparison of baseline reaction time for congruent and incongruent trials in the current study allowed for the classification of participants into one of two categories; attentional biasers or attentional avoidancers. Slower reaction times in congruent trials compared to incongruent trials was considered to be evidence of attentional avoidance at baseline; whereby faster reaction times in congruent compared to incongruent trials was considered evidence of attentional bias towards threat. Following this classification, chi-squared analyses revealed that there was an equal spread of males and females in both categories. Consequently, approximately 50% of both females and males displayed an attentional bias towards threat, and approximately 50% of females and males displayed an attentional avoidance effect,

therefore any attentional biases effects were likely cancelled out. There were no sex differences observed in those who reported an attentional bias compared to an attentional avoidance response.

As previously discussed, reaction time as a dependent variable lacks the sensitivity to discriminate covert attentional processes (Schmukle, 2005; Staugaard, 2009; Cisler & Koster, 2010). This may contribute to the lack of condition effects observed from reaction time data analysis measured during the dot-probe task. As such, ERP measures were included in the current study as an additional measure of attentional processes. ERPs are able to record shifts in attention usually missed in reaction time measures, with millisecond precision (Kapperman, et al., 2014).

Attentional Bias Effects in ERP Data

It was hypothesised that attentional avoidance to threat would be observed at baseline in females. This would be inferred from decreased amplitude in congruent conditions for all ERP components compared to incongruent and neutral conditions. Additionally, it was hypothesised that females would demonstrate attentional bias to threat post-stress induction which was measured by increased ERP amplitudes to congruent trials, compared to males. No condition effects or interactions between sex and condition, or sex and condition x time interactions (evidence of attentional bias or attentional avoidance to threat) were observed for P1, N1 or P3 ERP amplitudes at any topographical site in the current study. These findings did not support the current hypothesis, nor replicate the results of Carr et al. (2015), who found evidence of attention avoidance at baseline, and significantly greater attentional bias post-stress induction, compared to males. However, evidence of attentional avoidance was evident

in P1 amplitudes regardless of gender, by which P1 amplitudes were greater for neutral conditions compared to incongruent conditions. Evidence for an avoidance effect, but not attentional bias, in the ERP data confirms a potential role of the long-stimulus duration in the study minimizing the potential for displaying an attentional bias effect.

An alternative, and highly likely explanation for the lack of condition effect may be related to the subjective picture rating of the IAPS images used in the current study for the dot-probe task. Although images were selected from the IAPS database of arousal and valence norms for being unpleasant and arousing, participants in the current study did not report the threatening IAPS images any less unpleasant than the neutral IAPS images, or any more arousing. Although IAPS images have better ecological validity compared to word stimuli (Mogg & Bradley, 1999), and this set of images have been used in previous studies that have elicited negative ratings (Nicholson et al., 2014; Gardener et al. 2013), surprisingly, those selected were not perceived as threatening enough to invoke significant attentional biases. In a dot-probe task, Koster el al. (2006) used two stimuli types; IAPS images that were ranked as high threat and images that were regarded at moderate threat. As predicted their sample rated the high threat images as very negatively valenced (M = 2.02) and moderately arousing (M = 5.79). Moderate threat images were rated as negatively valenced (M = 3.10) and moderately arousing (M = 4.76), which were similar to the IAPS picture ratings of the current study. Results indicated that high threat images captured attention for all participants, but in the low-trait anxiety sample attentional bias was only observed for high threat images and attentional avoidance was evident for moderate threat images. In the high-trait anxiety sample, attentional bias was revealed for both high and moderate threat images, but it

was significantly greater towards high threat images. This effect has been labelled the attentional function account, by which high-trait anxiety individuals will attend to both moderate and high threat, whereas low-trait anxiety individuals will only attend to stimuli with high threat levels (Mogg & Bradley 1998). The results of the current study support the attentional function account, as it used a non-clinically anxious sample and moderate threat images and found no attentional bias effect. Mogg, McNamara, Powys, Rawlinson, Seiffer, et al. (2000) used high threat and mild threat images in a dot-probe task. Results indicated that attentional bias was evident for both high and moderate threat images, however high threat images evoked a significantly stronger attentional bias to threat. As such, it is suggested that a manipulation of image rating for valence and arousal should be considered in future studies.

Limitations and Future Research Implications

A key limitation of the current study was the lack of attentional bias or avoidance effect observed in reaction time and ERP amplitudes which failed to replicate previous studies (Carr et al., 2015; Kappenman et al., 2014). As outlined above, several methodological factors may explain this, and future research needs to build in specific modifications. In particular, the manipulation of stimuli duration time (to include both a rapid (200ms) and longer (>1000ms), IAPS images with a greater negative valence and higher arousal ratings should be included based on normative data (within the bounds of ethical constraints), and inclusion of a control group whom are not exposed to acute stress should all be taken into account

Menstrual phase has been shown to affect emotional memory function (Andreano, Arjomandi, & Cahill, 2008) and other cognitive functions (Mclean & Anderson, 2009).

The effect of menstrual phase may explain some of the variance of arousal and behavioural responses in the current sample. As part of saliva analysis, oestrogen and progestogen levels for each participant were calculated. Analysis of this data was out of the scope of the current honours project, but this information could be used later to help to control for menstrual phase in females. Furthermore, the stress hormone cortisol has been shown to interact with noradrenaline to affect attentional bias (Kreher et al., 2012), future research should analyse measures of cortisol with in addition to noradrenaline. Cortisol levels were not measured in the present study as peak latency would have required participants to wait and additional 30 minutes after acute-stress induction before completing the second dot-probe task. This would have added additional time to an already lengthy experimental time.

Due to the restrictions imposed by ethics, IAPS images selected as threat images were only moderately negative so as not to cause participants any undue distress. As discussed previously, participants in the current study did not rate the threat arousal and valence of IAPS images as anymore arousing or negative compared to neutral IAPS images. This may be a key reason for the failure to find an attentional bias effect. Stronger negative valence and high arousal IAPS images, coupled with shorter duration times, should be considered for future research to try and elicit more robust attentional bias affects.

Further to the limitation imposed by ethics, a non-clinical (anxiety) sample was used. There is an abundance of research indicating that attentional bias is a robust finding in clinically anxious samples (Mogg, et al., 2004; Koster et al., 2005; Koster et

al., 2006; Tan et al., 2011). As such, sex differences in attentional bias should be investigated in clinically anxious populations.

Additionally, the current study did not include a control group who did not complete the CPS task. To further the investigation of heightened arousal on reaction task, and to allow for the analysis of practice effects, a sample who were required to placed their hand in a bucket of luke-warm water, rather than ice water, should be considered.

Finally, post-hoc power analysis indicated that the current study was sufficiently powered (approaching .8; Field, 2013) for all significant results, and were approaching adequacy for those results trending towards being significant. Effects sizes for non-significant results in the current study were generally very small and can explain why observed power was so low. However, interpretation of the current findings should be done so with caution.

Future research should continue to explore the effect of sex differences on attentional bias as this was the first ERP study to do so whilst also inducing acute stress. Conclusions cannot be drawn regarding potential sex differences in attentional bias in the present study, as no valid attentional bias effects in reaction time or ERP measures were revealed. This may be due to lack of sufficiently high arousal and negative valence in IAPS images. Furthermore, it could potentially be related to high individual variability in attentional bias or avoidance which may be due to long stimuli duration times and composition of sample (non-clinical).

Conclusion

In conclusion, the present study examined sex differences in attentional bias to threat before and after threat whilst recording noradrenaline levels, reaction time, and ERP amplitude. The CPS elicited a significant increase in noradrenaline for both females and males, with males having greater tonic arousal and reactivity to acute stress compared to females. This was not consistent with the current hypothesis. In response to threatening IAPS images in a dot-probe task, no sex difference in reaction time or N1 and P3 ERP amplitudes were observed, which did not support the hypotheses. However, larger P1 amplitudes were observed for neutral compared to incongruent dot-probe conditions, which may be indicative of attentional bias. No conclusions about attentional bias and its effects of female anxiety disorder prevalence can be made based on these findings. Future research should consider the manipulation of methodological factors such as including shorter stimulus duration times, and select IAPS images with more negative valence and higher arousal ratings to try and elicit more robust attentional bias or avoidance effects.

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Table of Appendices

Appendix A

Attentional Bias to Threat: ERP, reaction time, and noradrenaline measures: Demographic & clinical data

Date: / / Time:

Participant assigned number:

Age: Sex:

Current medications, including contraceptive pill or other forms of hormonal contraceptive (i.e. Implanon, Nuva Ring, Mirena):

Any previously or currently diagnosed psychological or medical disorders (including traumatic or acquired brain injury, learning difficulties, or attentional deficits etc.):

TEQ Score: PCL-5 Score:

DASS-21: Total Score: D: A: S:

Assigned group: TE / NTE

Handedness:

Time spent immersed in cold pressor task:

Appendix B

Social Science Ethics Officer
Private Bag 01 Hobart
Tasmania 7001 Australia
Tel: (03) 6226 2763
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Human.ethics@utas.edu.au

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

13 April 2015

Assoc Prof Kim Felmingham
Psychology
Private Bag 30

Sent via email

Dear Assoc Prof Felmingham

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0012494 - Trait Social Anxiety and Emotional Processing: An ERP Study**

- Change to student investigators: addition of Lauren Reading and Emma Jackson, removal of Laura Stewart.
- Addition of investigator Dr Allison Matthews.
- Extend data collection to both male and female participants.
- Change of task to use emotional stimuli from the International Affective Picture Series.
- Additional brief self-report measure of previous traumatic life experiences, the Traumatic Life Events Questionnaire.
- Revised Information Sheet.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 9/4/2015.

Yours sincerely

Appendix C

DASS21

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

Appendix D

TRAUMATIC EXPERIENCE

Below is a list of very traumatic or upsetting events that sometimes happen to people. Please indicate if any of these events have happened to you:

1. Have you ever had direct combat experience in a war? Yes ☐
2. Have you ever been involved in a life-threatening accident? Yes ☐
3. Have you ever been involved in a fire, flood or other natural disaster? Yes ☐
4. Have you ever witnessed someone being badly injured or killed? Yes ☐
5. Have you ever been seriously attacked, assaulted or molested? Yes ☐
6. Have you ever been threatened with a weapon, held captive, or kidnapped? Yes ☐
7. Have you ever been tortured or the victim of terrorists? Yes ☐
8. Have you ever experienced an extremely stressful or upsetting event? Yes ☐
9. Have you ever suffered a great shock because one of the events on the list happened to someone close to you? Yes ☐

If you are happy to be contacted for potential participation in a research study related to this questionnaire, please write your contact details below:

Name:_____

Email: _____

Mobile: _____ ¶

Appendix E

PCL-5

Instructions: Below is a list of problems that people sometimes have in response to a very stressful experience. Please read each problem carefully and then circle one of the numbers to the right to indicate how much you have been bothered by that problem in the past month.

In the past month, how much were you bothered by:	*Not at all*	*A little bit*	*Moderately*	*Quite a bit*	*Extremely*
1. Repeated, disturbing, and unwanted memories of the stressful experience?	0	1	2	3	4
2. Repeated, disturbing dreams of the stressful experience?	0	1	2	3	4
3. Suddenly feeling or acting as if the stressful experience were actually happening again *(as if you were actually back there reliving it)*?	0	1	2	3	4
4. Feeling very upset when something reminded you of the stressful experience?	0	1	2	3	4
5. Having strong physical reactions when something reminded you of the stressful experience *(for example, heart pounding, trouble breathing, sweating)*?	0	1	2	3	4
6. Avoiding memories, thoughts, or feelings related to the stressful experience?	0	1	2	3	4
7. Avoiding external reminders of the stressful experience *(for example, people, places, conversations, activities, objects, or situations)*?	0	1	2	3	4
8. Trouble remembering important parts of the stressful experience?	0	1	2	3	4
9. Having strong negative beliefs about yourself, other people, or the world *(for example, having thoughts such as: I am bad, there is something seriously wrong with me, no one can be trusted, the world is completely dangerous)*?	0	1	2	3	4
10. Blaming yourself or someone else for the stressful experience or what happened after it?	0	1	2	3	4
11. Having strong negative feelings such as fear, horror, anger, guilt, or shame?	0	1	2	3	4
12. Loss of interest in activities that you used to enjoy?	0	1	2	3	4
13. Feeling distant or cut off from other people?	0	1	2	3	4
14. Trouble experiencing positive feelings *(for example, being unable to feel happiness or have loving feelings for people close to you)*?	0	1	2	3	4
15. Irritable behavior, angry outbursts, or acting aggressively?	0	1	2	3	4
16. Taking too many risks or doing things that could cause you harm?	0	1	2	3	4
17. Being "superalert" or watchful or on guard?	0	1	2	3	4
18. Feeling jumpy or easily startled?	0	1	2	3	4
19. Having difficulty concentrating?	0	1	2	3	4
20. Trouble falling or staying asleep?	0	1	2	3	4

PCL-5 (8/14/2013) Weathers, Litz, Keane, Palmieri, Marx, & Schnurr – National Center for PTSD

Appendix F

Attentional Bias to Threat: ERP, reaction time, and noradrenaline measures. Participant Information Sheet

1. Invitation

Thank you for your interest in this research. This study is being conducted in partial fulfilment of a Psychology Honours degree for Lauren Reading and Emma Jackson under the supervision of Professor Kim Felmingham, Dr Andrea Carr, and Dr Allison Matthews at the University of Tasmania. Please take your time to read this information sheet to gain a better understanding of the research task and what it will involve. Before you decide to participate, it is important that you understand all of the information below. If you have any further questions or would like more information please contact the researchers at lreading@utas.edu.au (Lauren Reading) or emmaj4@utas.edu.au (Emma Jackson).

2. What is the purpose of this study?

The study aims to assess attentional biases to threat at before and after inducing an acute stress task in individuals who have been exposed to trauma compared to individuals without trauma exposure. The results from this study will be used to inform further research in the area of trauma exposure and PTSD.

3. What will I be asked to do?

As a participant you will be asked to complete a dot-probe task on a computer, where neutral and threatening images will be presented and your reaction time to a dot appearing on screen after each pair of images will be used to assess attentional bias. You will be required to wear an EEG cap so that your brainwaves can be recorded to further assess your attentional bias and response.

You will be required to undergo a cold-pressor stress task, where you will need to immerse one of your hands into a bucket of water maintained below 4 degrees Celsius for a maximum of three minutes. This may be uncomfortable but will not cause injury and is intended to cause a stress response in the body. You will be also required to give a saliva sample before the dot-probe begins and then after the cold-pressor stress task, to enable measurement of the stress hormone noradrenaline in your system.

4. Are there any possible benefits from participation in this study?

Your participation will promote further research, providing valuable information to clinicians and researchers working with a variety of clients.

5. Are there any possible risks from participation in this study?

This study involves no more than minimal risk (i.e. risks encountered in daily life) and no specific risk is anticipated with taking part in this study. The cold-pressor stress task may be uncomfortable but will not cause injury, and there is a slight risk of skin irritation from the products used to prepare your skin for the placement of the EEG cap. No deception is involved in this study. At any time should you feel uncomfortable or upset completing the tasks involved, please stop the task and approach the researcher.

6. What if I change my mind during or after the study?

Your involvement in the study is completely voluntary and you are able to withdraw at any time without negative consequence. However, please note that after you have completed your testing we will not be able to remove your data from the data-set as there is no way of knowing which responses belong to you, as the data is de-identified.

7. Anonymity

As mentioned above, all data recorded in this experiment will be de-identified, meaning that there is no way to identify who has participated or link any information or scores back to a participant. Participants are assigned a number and their data is stored under that, there is no link between their identity and this number, it is purely a way to separate different participants' information.

8. What will happen to the information when this study is over?

The data relating to the study will be encrypted and stored in a secure, password-protected electronic database on the University of Tasmania, School of Medicine (Psychology) premises. Your name will not be recorded or associated with any experimental data.

The research data will be stored for the minimum of five years. After five years from the date of the first publication all data will be deleted within the formal guideline of the University of Tasmania' data destruction processes.

9. How will the results of the study be published?

The findings of this study will be available at the University of Tasmania website http://www.utas.edu.au/psychology/ or can be requested via email. For further information please contact Lauren Reading at email lreading@utas.edu.au or Emma Jackson at email emmaj4@utas.edu.au. The results will be published as a thesis by both researchers, and may possibly be published by a scientific journal if important findings are made.

10. What if I have questions about this study?

If you have any further questions about this study, please contact Lauren Reading (student researcher) at lreading@utas.edu.au or Emma Jackson (student researcher) at emmaj4@utas.edu.au or Kim Felmingham (Chief Investigator) at Kim.Felmingham@utas.edu.au.

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on +61 3 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number H0012494.

Thank you for your time taken reading this information sheet.

Appendix G

Consent form for experimental participants

Attentional bias to threat: ERP, reaction time, and noradrenaline measures

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. I understand that the study involves viewing images which may be threatening in nature, immersing my hand in ice-water for up to three minutes, and giving two saliva samples that will be used only to assess the level of the hormone noradrenaline present in my body. I understand that completion of participation in this study will take approximately two hours of my time.
5. I understand that participation involves the risk(s) that I may be upset by the threatening images presented. If this occurs, I understand that the researcher can refer me to the University Psychology Clinic for counselling.
6. I understand that all research data will be securely stored on the University of Tasmania's premises for five years from the publication of the study results, and will then be securely destroyed.
7. Any questions that I have asked have been answered to my satisfaction.
8. I understand that the researcher(s) will maintain confidentiality and that any information I supply to the researcher(s) will be used only for the purposes of the research.
9. I understand that the results of the study will be published in a manner so that I cannot be identified as a participant.
10. I understand that my participation is voluntary and that I may withdraw at any time without any effect. I understand that I will not be able to withdraw my data after completing the experiment as the data has been de-identified and cannot be linked back to me.

Participant's name:

_____

Participant's signature:

_____

Date: _____

Statement by Investigator

☐ I have explained the project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have had the opportunity to contact me prior to consenting to participate in this project.

Investigator's name:

_____

Investigator's signature:

_____

Date: _____

G

Appendix H

Instructions for dot-probe task

- Please sit comfortably upright, with your face 50cm from the screen of the computer. The experimenter will help you place your chair correctly to facilitate this.
- Place your hands on the keyboard, with your index fingers on the A and L keys. **Pressing A indicates left, pressing L indicates right**.
- For each trial, a pair of images will be presented for 1 second. Please look at the images.
- The images will disappear and be replaced by a white dot on the left or right.
- You will need to press the key that corresponds to the same side of the screen as the dot as fast as you can once you see the dot.

  Again, **the A key indicates left, the L key indicates right**
- The next set of images will appear once you have done this, with 57 trials in total, taking approximately 7.5 minutes to complete.
- **Please keep as still as possible, breathe and blink normally, and do not worry about any mistakes you may make, just be ready for the next trial when it appears.**
- **If you have any questions, please ask the experimenters**.

Thank you again for your participation.

Appendix I

Table 2. *Non-significant main effect and interaction for Reaction Time*

Variable	*df*	*F*	*p*	η_p^2	λ
Condition	2, 34	.40	.67	.02	.98
Condition x Sex	2, 34	.99	.38	.06	.95
Time x Sex	1, 35	.23	.63	.01	.99
Condition x Time	2, 34	1.26	.30	.07	.93
Condition x Time x Sex	2, 34	.45	.64	.03	.97

Appendix J

Table 3. *Non-significant main effects and interactions for P1 ERP Component*

Variable	*df*	*F*	*p*	η_p^2	λ
Sex	1, 35	.04	.84	.00	-
Time	1, 35	.95	.34	.02	-
Condition x Sex	2, 34	.43	.66	.02	.98
Time x Sex	1, 35	.00	.99	.00	1.00
Site x Sex	2, 34	.76	.47	.04	.96
Time x Site	4, 32	1.34	.28	.07	.93
Condition x Site	4, 32	1.26	.31	.14	.86
Condition x Time	2, 34	.58	.56	.03	.58
Condition x Time x Sex	4, 34	2.03	.15	.12	.89
Condition x Site x Sex	4, 32	1.77	.16	.18	.82
Time x Site x Sex	2, 34	.23	.79	.01	.99
Condition x Time x Site	4, 32	.22	.93	.03	.90
Condition x Time x Site x Sex	4, 32	.86	.50	.10	.90

Appendix K

Table 4. *Non-significant main effects and interactions for N1 ERP Component*

Variable	*df*	*F*	*p*	η_p^2	λ
Condition	2, 34	2.33	.11	.12	.88
Condition x Sex	2, 34	2.59	.09	.13	.87
Time x Sex	1, 35	.07	.79	.00	1.00
Site x Sex	2, 34	.56	.58	.03	.97
Time x Site	2, 34	.25	.78	.01	.99
Condition x Site	4, 32	1.18	.34	.13	.87
Condition x Time	2, 34	1.28	.29	.07	.93
Condition x Time x Sex	2, 34	.41	.54	.02	.98
Condition x Site x Sex	4, 32	.94	.45	.11	.89
Time x Site x Sex	2, 34	1.66	.21	.09	.91
Condition x Time x Site	4, 32	.79	.54	.09	.91
Condition x Time x Site x Sex	4, 32	1.03	.41	.11	.89

Appendix L

Table 5. *Non-significant main effects and interactions for P3 ERP Component*

Variable	*df*	*F*	*p*	η_p^2	λ
Sex	1, 35	1.85	.18	.05	-
Time	1, 35	1.59	.22	.04	.96
Condition	2, 34	.90	.91	.00	1.00
Condition x Sex	2, 34	1.29	.29	.07	.93
Time x Sex	1, 35	.03	.87	.00	1.00
Time x Site	2, 34	1.29	.29	.07	.93
Condition x Site	4, 32	2.03	.11	.20	.93
Condition x Time	2, 34	.54	.59	.03	.97
Condition x Time x Sex	2, 34	.19	.83	.01	.99
Condition x Site x Sex	4, 32	.33	.86	.04	.96
Time x Site x Sex	2, 34	.30	.74	.02	.98
Condition x Time x Site	4, 32	.35	.84	.04	96
Condition x Time x Site x Sex	4, 32	1.19	.35	.13	.03

Appendix M

Table 6. *Summary of attentional bias research using dot-probe tasks*

Author	Stimuli Type	Stimuli Duration (ms)	Result
Mogg (1997)	Words	100, 500, 1500	AB in all conditions; strongest in 100 group
MacLeod, et al. (1986)	Words	500	AB in clinically anxious group
Lipp, et al. (2005)	IAPS images	500	AB
Koster et al., (2006)	IAPS images	500	AB in HTA group
Koster et al., (2005)	IAPS images	100, 500, 1250	AB in HTA group of 100 & 500; AA in 1250
Salemink (2007)	Word	500	Disengagement, no orientation to threat
Eldar et al., (2010)	Faces	500	AB in anxious individuals
Hunt (2006)	Words	500	AB in anxiety sensitive sample
Cooper et al., (2006)	Faces	100, 500	AB in 100; AA in 500
Koster et al., (2004)	IAPS images	500	AB
Bradley et al., (1998)	Faces	500, 1250	AB in HTA sample; non-significant trend in 1250
Tan et al., (2011)	Faces	800, 1250, 1500	HTA females AB in 800; HTA male AA
Tran et al., (2013)	Faces	50	AB in anxious females
Bradley et al., (1997)	Faces	500	No effect
Mansell et al., (1999)	Faces	500	HSA group AA
Britton et al., (2015)	Faces	500	No effect

AB = Attentional Bias, AV = Attentional Avoidance, HTA = High Trait Anxiety, HAS = High State Anxiety

Appendix N

SPSS databases and outputs

1. Correlation Analysis sAA and Reaction Time Output
2. Demographic Reaction Time PCL DASS MEDS SAA database
3. ERP N1 database
4. ERP P1 database
5. ERP P3 database
6. IAPS picture rating Arousal
7. IAPS picture rating Valence
8. IAPS picture rating Valence database
9. IAPS picture rating Arousal database
10. Repeated Measures ANOVA Reaction Time
11. Repeated Measure ANOVA N1 FCz Cz
12. Repeated Measure ANOVA P1 Pz P3 P4
13. Repeated Measure ANOVA P3 Cz CPz FCz
14. sAA ANOVA Sex
15. Sex difference DASS PCL ANOVA